

PRIMARY STRUCTURE OF RAT LIVER Z-PROTEIN

A low- M_r cytosol protein that binds sterols, fatty acids and other small molecules

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1. Introduction

In the cytosol of animal tissues, Z-protein [1] (fatty acid-binding protein [2] or squalene- and sterol-carrier protein [3]) binds free fatty acids, their CoA derivatives, bilirubin, organic anions and other small molecules [4]. It appears to play a significant role as a carrier protein in reversing the inhibitory effect of palmitoyl CoA on acetyl CoA carboxylase [5] and on adenine nucleotide transport of isolated mitochondria [6]. A possible role of Z-protein in fatty acid metabolism has been suggested by the findings that it stimulated microsomal acyl CoA:glycerophosphate acyl-transferase [7] and mitochondrial β -oxidation of fatty acids [8].

A number of cytosolic proteins, such as supernatant protein factor [9], phosphatidylcholine exchange protein [10], nonspecific phospholipid exchange protein [11] and retinol-binding protein [12] have been reported for their critical roles in intracellular lipid transport and metabolism. Some of them show a close similarity with respect to molecular size, amino acid composition and affinity to lipids, suggesting possible structural relationships among these proteins. In order to investigate the structural bases for their relationship and biological activities, we have purified Z-protein from rat liver and determined its primary structure. Here, we present the complete amino acid sequence of Z-protein, which showed partial similarities with the reactive regions of acyl-carrier protein [13] and phosphatidylcholine exchange protein [10].

2. Materials and methods

2.1. Isolation of rat liver Z-protein

The supernatant fractions were prepared from

homogenates of rat livers in 0.1 M Tris-HCl (pH 7.4) with 1 mM EDTA by centrifugation at $105\,000 \times g$ for 60 min. The supernatant obtained from fractionation with 70% saturated ammonium sulfate was dialyzed against 30 mM Tris-HCl (pH 8.5), concentrated by ultrafiltration (Amicon UM-10) and applied to a column of Sephadex G-75. The Z-protein fraction binding indocyanine green (Daiichi Seiyaku) was applied to a DEAE-cellulose column equilibrated with 30 mM Tris-HCl (pH 8.5).

2.2. Amino acid analysis

Protein (0.2–0.5 mg) and peptide (5–50 nmol) samples were routinely hydrolysed with 5.7 N HCl for 24 h at 110°C under vacuum, and analysed on a Hitachi KLA 3B or a JEOL JLC-6AS amino acid analyser.

2.3. Sequence analysis

Sequence of protein or peptides was analysed either by a JEOL JAS 47K sequenator [14] or by the manual Edman degradation method [15]. Phenylthiohydantoin derivatives were identified by thin-layer chromatography on Kieselgel 60 F-254 sheets (Merck) [16] and by the back hydrolysis method [17]. COOH-terminal sequence was determined by digestion with carboxypeptidase A and *Penicillium* acid carboxypeptidase [18].

2.4. CNBr cleavage and separation of peptides

The protein was treated with a 100 fold molar excess of CNBr over methionine residues in 70% formic acid for 24 h at 20°C. The CNBr fragments were purified by gel filtration on a Sephadex G-50 column (1.9 \times 140 cm), and the smaller fragments were further purified by high voltage paper electrophoresis.

2.5. Enzymatic digestion and hydroxylamine cleavage

The larger CNBr peptides were further digested with trypsin. To provide overlaps for the CNBr fragments, digestion of performic acid-oxidized Z-protein with trypsin or *Staphylococcus aureus* V-8 protease [19] and cleavage at Asn-Gly by hydroxylamine [20] were used.

3. Results and discussion

3.1. Isolation of Z-protein

Z-protein was adsorbed weakly to the DEAE-cellulose and separated into two peaks (DE-I, DE-II) by elution with the starting buffer. The two fractions of Z-protein respectively gave a single band with an M_r -value of 14 000 by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and 2-mercaptoethanol. The amino acid compositions of DE-I and DE-II are almost identical (table 1) and similar to those of squalene- and sterol-carrier protein [3], aminoazodye-binding protein A [21] and Z-protein [22]. DE-II was used for the sequence analysis in this study.

Table 1
Amino acid compositions of DE-I and DE-II^a

	DE-I	DE-II
Cys(Cm) ^b	0.8	0.7
Asp	11.1	10.7
Thr ^c	10.0	11.3
Ser ^c	4.5	5.8
Glu	17.0	17.2
Pro	1.8	1.5
Gly	11.4	11.5
Ala	2.6	2.2
Val ^d	13.2	11.5
Met	5.7	6.7
Ile ^d	9.4	8.6
Leu	6.4	6.0
Tyr	2.8	2.8
Phe	5.9	5.8
Lys	16.5	17.2
His	1.7	2.1
Arg	1.7	2.1

^a An M_r of 14 000 was used to calculate amino acid compositions

^b Determined as S-carboxymethylcysteine

^c Determined by extrapolation to initial time of hydrolysis

^d Determined assuming complete release after 72 h hydrolysis

3.2. NH₂- and COOH-terminal sequence of Z-protein

Sequenator analysis on 3.0 mg oxidized Z-protein revealed the NH₂-terminal sequence as Met(O₂)-(Asn-Phe-Ser-Gly-Lys-Tyr-. The yield of PTH Met(O₂) was below 16% of the starting material, suggesting that most of NH₂-terminal Met was blocked. The COOH-terminal sequence was identified as -Lys-Arg-Ile by *Penicillium* acid carboxypeptidase digestion.

3.3. Sequence of CNBr-fragments

Seven CNBr-fragments were obtained by gel filtration followed by paper electrophoresis. The sum of their amino acid compositions accounted for the total composition of Z-protein except for one Met residue (data not shown). This was recovered as blocked homoserine lactone from the pass through fraction of another CNBr digest from an SP-Sephadex C-25 column (0.05 M pyridine-formate, pH 2.4). Although this protein was resistant to digestion with an acetyl amino acid-releasing enzyme [23], the blocked amino acid was identified as *N*-acetylhomoserine lactone by high pressure liquid chromatography and thin-layer chromatography [24]. Sequence of these CNBr fragments and their order were determined as summarized in fig.1.

The primary structure of Z-protein indicates the presence of a clustering of glutamic acids in the middle part of the molecule. A sequence repetition at residues 33-40 and 96-103 is also observed (fig.2a). Comparison of the sequence of Z-protein with those of other proteins showed partial similarities with *Escherichia coli* acyl-carrier protein [13], acyl-carrier protein component of rat liver fatty acid synthetase [25], and bovine phosphatidylcholine exchange protein [10]. As seen in fig.2b, the sequence of residues 65-81 of Z-protein shows some similarity with those around the 4'-phosphopantetheine-bound serines of the acyl-carrier proteins. The predictive models [26] for the secondary structure of these segments are equally long α -helices. The presence of the sole thiol group of Z-protein (Cysteine 69) in this region appears to be indicative in analogy with the thiol group of 4'-phosphopantetheine in the acyl-carrier proteins, though nothing is known about the function of the thiol in Z-protein.

Amino acid sequence around the binding site for the fatty acyl moiety in phosphatidylcholine exchange protein was proposed using the photoaffinity labelling technique [27]. A peptide segment having the similar sequence (fig.2c) to this binding site may also form

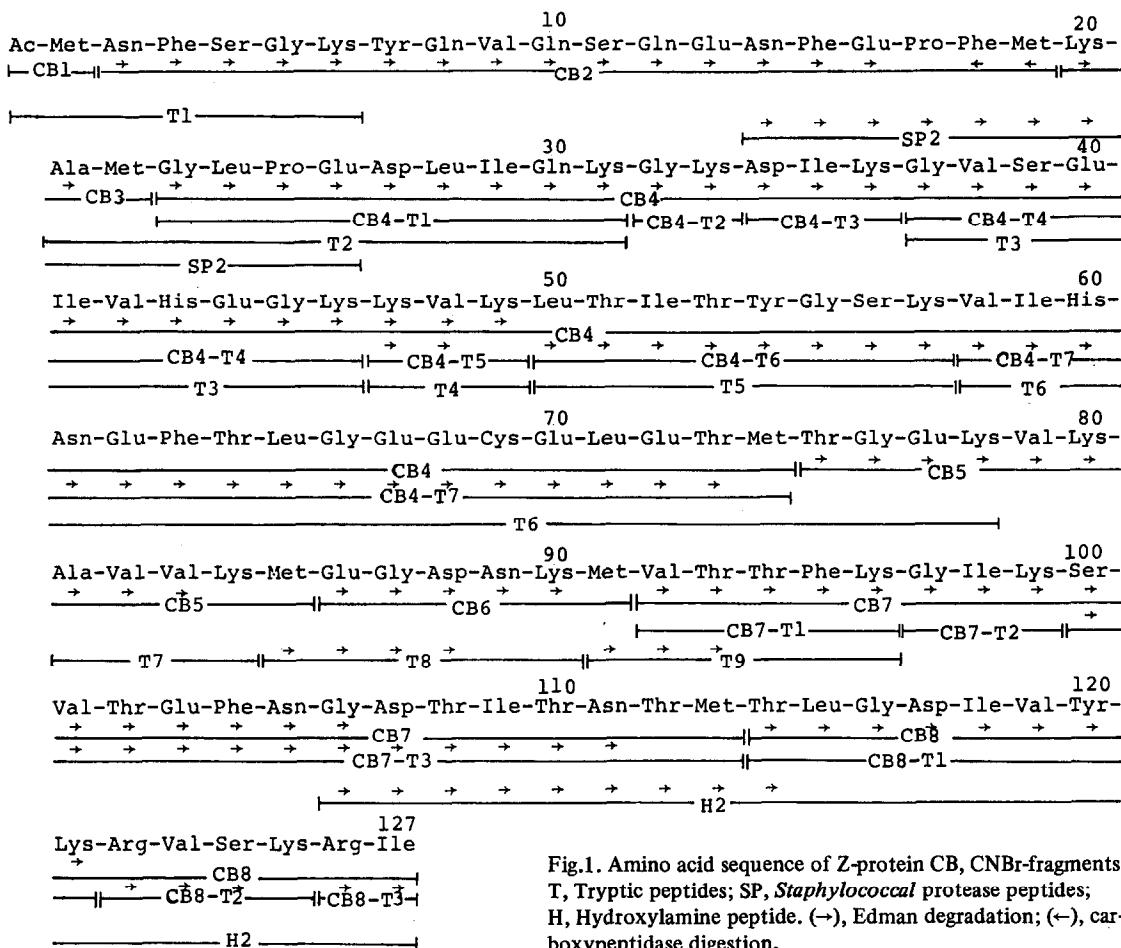
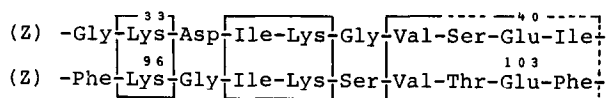


Fig.1. Amino acid sequence of Z-protein CB, CNBr-fragments; T, Tryptic peptides; SP, *Staphylococcal* protease peptides; H, Hydroxylamine peptide. (→), Edman degradation; (↔), carboxypeptidase digestion.

a.



b.

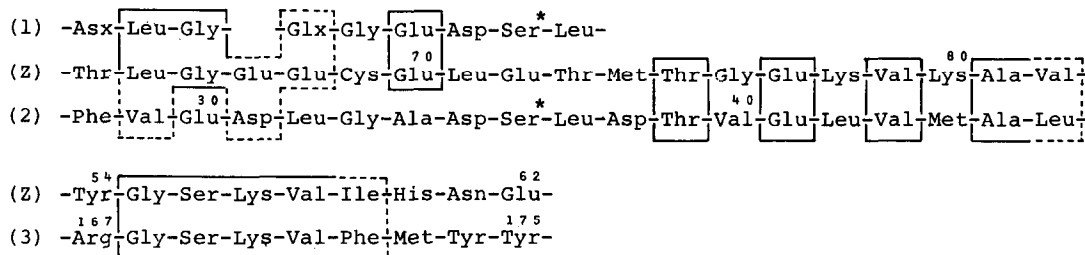


Fig.2. Repetitive sequence in Z-protein (a) and comparison of the sequence of Z-protein (Z) with those of other proteins (b). (1) 4'-phosphopantetheine peptide from acyl-carrier component of rat liver fatty acid synthetase [25]. (2) *Escherichia coli* acyl-carrier protein [13]. (3) Binding site peptide of phosphatidylcholine exchange protein [27]. Residues identical or conservative with Z-protein are in boxes. Gap was included to maximize the degree of homology. * Serine to which the prosthetic group, 4'-phosphopantetheine, is bound.

one of the fatty acid-binding sites in Z-protein. Although significance of these short sequence similarities is uncertain, it is tempting to propose that these polypeptide segments constitute several functional domains responsible for the reported multiplicity of Z-protein functions.

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